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Maximum Urine Concentrating Ability in Children With Hb SC Disease: Effects of Hydroxyurea

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Studies in adults with Hb SC disease suggested that hydroxyurea reduced hemolysis and increased red cell hydration. Because increased hydration should diminish the polymerization tendency of Hb S we hypothesized that hydroxyurea might repair the urine concentration defect of HbSC disease. Eight Hb SC disease patients, aged 10 to 17 years, were given hydroxyurea daily. Maximal urine concentrating ability following overnight fasting and after subcutaneous arginine vasopressin (dDAVP), blood counts, and cell volumes were observed for 12–15 months. All patients had impaired urine concentrating ability prior to hydroxyurea treatment and failed to increase their ability to concentrate urine following treatment (maximum urine concentration after an overnight fast and dDAVP, 520–530 mOsm). Mean corpuscular volume (MCV) and reticulocyte MCV increased after administration of hydroxyurea, and the reticulocyte count and ratio of red cell hemoglobin to reticulocyte hemoglobin fell but there was little change in PCV. Hb F increased substantially in 2 patients but showed little change in the remaining patients. There was no evidence that hydroxyurea was associated with increased urine concentrating ability in children with Hb SC disease. These results may reflect irreversible renal medullary damage prior to beginning treatment or insufficient intensity or duration of treatment. Am. J. Hematol. 64:47–52, 2000.

Key words: hemoglobin SC disease; hemoglobin C; sickle hemoglobin; hydroxyurea; dense cells; hyposthenuria

In Hb SC disease, erythrocytes have reduced cation content and increased density because of the presence of both Hb S and Hb C [1–4]. Rehydration of Hb SC cells reduced their oxygen affinity, rate of sickling, deoxygenation-induced K^+ efflux, and mean cell hemoglobin concentration (MCHC) [1,2,5], suggesting that decreasing the density of these cells might alleviate the pathology associated with this disease [5].

Hydroxyurea (HU), an approved treatment for sickle cell anemia, may exert its therapeutic benefits by mechanisms beyond its effect on fetal hemoglobin (Hb F) [6–13]. In adults with Hb SC disease, HU was associated with advantageous cellular changes; hemolysis and "stress" erythropoiesis decreased and cell hydration improved [14].

Among the many abnormalities of sickle cell disease is the effect of sickling on renal concentrating ability [15]. An inability to concentrate urine normally is apparent in sickle cell anemia, Hb SC disease, and even in the sickle cell trait. Urine concentrating ability is lost early in sickle cell anemia, late in sickle cell trait, and intermediate between these entities in Hb SC disease [16,17]. In sickle cell anemia, an early functional concentrating defect—reversible by transfusion of normal red cells—with time is converted to anatomic damage to the renal medulla and

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TABLE I. Effect of Hydroxyurea on Maximal Urine Concentrating Ability (mOsm/kg Water) in Children With Hb SC Disease

	Pre-drug	3 months	6 months	12 months	15 months
Baseline	446 ± 36	451 ± 27	428 ± 30	389 ± 15	387 ± 33
2 hr post dDAVP	493 ± 36	482 ± 36	488* ± 26	443 ± 28	457 ± 28
4 hr post dDAVP	526* ± 31	493 ± 39	508* ± 37	461* ± 23	477* ± 34
N	8	8	8	7	7

*$P < 0.05$ versus pre-drug.

a permanent inability to concentrate urine. Pertinent to the role of cell density and Hb S polymerization in determining the concentrating defect in sickle cell trait, we showed that maximal urine concentrating ability was dependent upon the presence or absence of α thalassemia, a disorder that affects the density of the red cell [18]. By reducing the polymerization tendency in sickle cell trait cells, α thalassemia may preserve the medullary hypertonicity and countercurrent mechanism required for urine concentration.

We hypothesized that HU, by reducing the polymerization tendency of Hb S, might improve the ability of young patients with Hb SC disease to concentrate urine. Accordingly, we treated children with Hb SC disease with HU and followed their ability to maximally concentrate their urine.

METHODS

Patients

Eight children with Hb SC disease, aged 10 to 17 years (mean age 11 years, 3 males, 5 females) were enrolled in this study which was approved by the Institutional Review Board of the University of Mississippi Medical Center. They were not transfused, had serum creatinine concentration less than 175 μmol/L, and serum ALT less than 45 IU/L.

Hydroxyurea Treatment

HU was given as a single daily dose of 15 mg/kg.

Laboratory Studies

Complete blood counts which were monitored using a Coulter® Counter, serum chemistries, Hb F level [19], and red cell cation content [20,21] were done at baseline before drug administration and repeated periodically during treatment.

Bayer H*3™ analysis, also done at baseline and repeated at intervals during treatment, included standard cell counts and red cell indices [22] and measurements of reticulocyte volume (MCVr), total reticulocyte hemoglobin (rHb), ratio of total hemoglobin to rHb (Hb/rHb), and absolute reticulocyte count and reticulocyte mean cell hemoglobin concentration (CHCMr). From the absolute reticulocyte count and the reticulocyte hemoglobin concentration, rHb, which expresses the hemoglobin content of all reticulocytes in g/L, is calculated [23]. The ratio of total red cell Hb to rHb defines the ratio between the hemoglobin contained in mature red cells and in the reticulocytes and is a measure of hemolysis.

Measurement of Maximal Urine Concentrating Ability

Before determining the maximal urine concentrating ability, the use of nonsteroidal anti-inflammatory drugs and aspirin were discontinued for at least 1 week. Subjects arrived at the clinic following an overnight fast, having been instructed to discard their first morning voided urine. Following the collection of the next spontaneously voided urine, 0.08 mcg/kg of Desmopressin Acetate (dDAVP, Rorer Pharmaceuticals) was given subcutaneously. Urine voided at 2 and 4 hr after dDAVP administration was collected. Urine osmolality was measured by freezing point depression using an Advanced Micro-Osmometer (Model 3MO: Advanced Instruments, Needham Heights, MO).

Statistics

The paired, 2-tailed Wilcoxon signed-rank test, comparing baseline with post-treatment values, was used to test for significance.

RESULTS

Urine Concentrating Ability

Eight patients were treated for a mean of 14 months (12–15 months). At baseline before beginning HU treatment and prior to receiving dDAVP, no patient had an overnight urine concentration greater than 545 mOsm (mean 446 mOsm; normal range 800–1000 mOsm). At the time of baseline measurements seven of eight patients increased their urine concentration after receiving dDAVP although none increased urine concentration to normal (Table I). In no patient did HU appear to increase the maximal urine concentration ability beyond that of the baseline value. Table I suggests that over the 12–15 months of study, there was a deterioration of the maximum urine concentrating ability.

Figure 1 shows the variations in maximal urine concentrating ability (mOsm), Hb F level, MCV, CHCMr,

Hb/rHb, and reticulocyte count over time in two patients with Hb SC disease treated with HU, one (Fig. 1A) with no change in Hb F level and another (Fig. 1B) whose Hb F increased from 6.3% to 15% during treatment.

Hb F

Two patients, a 10-year old girl and a 17-year old girl, increased their concentration of Hb F during treatment with HU. The first patient had an increase from 6.3% to between 14% and 15% Hb F after 2–3 months of treatment (Fig. 1B). A second patient increased her Hb F from 1% to about 6% after 5–6 months of treatment. No other patient had an increase of Hb F beyond 1.5% (Figure 2).

Cell Volumes, Cell Hemoglobin, Cell Counts

MCV increased from 73.5 ± 8.1 fL at baseline to 83 ± 12.5 fL at the conclusion of treatment ($P = 0.018$), with a corresponding increased in MCVr from 87.4 ± 6.3 to 101 ± 8.7 fL ($P = 0.028$). PCV, 31.9 ± 1.9 at baseline, did not change significantly (32.5 ± 1.3; $P = 0.21$) and MCHC, 36.3 ± 1.1 g/dL at baseline, fell slightly but not significantly (35.5 ± 1.4 g/dL; $P = 0.069$).

Absolute reticulocyte count fell from (171.5 ± 49.5) × 10^9/L pretreatment to (123.3 ± 44.5) × 10^9/L at the conclusion of data collection ($P = 0.017$), suggesting either a reduction of hemolysis or suppression of erythropoiesis. Under steady-state conditions, erythrocyte survival may also be estimated indirectly from the ratio of hemoglobin contained in mature red cells and in reticulocytes [23]. The ratio of Hb/rHb at the baseline (29.5 ± 11.2) and after treatment (37.3 ± 14.2) also suggested some prolongation of red cell survival.

Chemistries and Toxicity

Serum creatinine and ALT were not affected by HU treatment. There were no complications of HU treatment, and in no patient did toxicity require cessation of treatment.

DISCUSSION

Although "milder" than sickle cell anemia, Hb SC disease is associated with considerable morbidity and increased mortality underscoring the need for an effective treatment [24–29]. Sickle hemoglobin polymerization within Hb SC cells is primarily due to their high hemoglobin concentration. Rehydrating these cells returned their abnormal properties toward normal [1–5]. We showed previously that HU was associated with a decline in hemolysis in adults with Hb SC disease and that there was a fall in the percent of dense red cells [14]. Reduced cell density should diminish the polymerization tendency of Hb S within Hb SC disease cells. We hypothesized that by reducing the polymerization tendency of Hb S, HU might improve and preserve the renal medullary countercurrent mechanism, thus increasing the ability of young patients with Hb SC disease to concentrate urine. Increased urine concentrating ability might provide a physiological marker of the clinical effectiveness of this drug in Hb SC disease.

Deterioration of renal concentrating ability in Hb SC disease has not been extensively studied. In 14 patients with Hb SC disease, ages 6 to 65 years (mean age 30 years), the maximum concentrating ability was 537 mOsm and appeared to decline with age [16]. The two youngest patients in that study, aged 6 and 8 years, had maximum urine concentrations of 640 and 698 mOsm, about 60% of normal. In children with sickle cell anemia under age 10 years, transfusion with normal red blood cells can reverse hyposthenuria, suggesting that renal medullary damage is reversible. Reversibility after transfusion is lost between ages 10 and 15 years due to structural damage in the renal medulla [30,31]. Our patients, whose average age was 11 years when treatment began, had pretreatment maximum urine concentrations of 525 mOsm—about half the expected normal value—suggesting an earlier than anticipated loss of renal medullary function. Within this small group of older children, treatment with HU did not increase the ability to concentrate urine. Urine concentrating ability failed to increase significantly in any patient, even when Hb F concentration increased. During the study period there even seemed to be a decrease in concentrating ability. Hemolysis appeared to be reduced in all patients, as estimated by the fall in reticulocyte count and the ratio of Hb/rHb yet there were no significant changes in PCV and the MCHC changed little. An increase in PCV is not necessarily a desirable goal for the treatment of Hb SC disease since much of the pathology of this disorder may result from increased blood viscosity caused by dense cells. In our previous study of hydroxyurea in adults with Hb SC disease we speculated that any adverse effect of the small but significant increase in PCV might be offset by the fall in red cell density [14].

To effect beneficially renal medullary function—and perhaps to forestall damage to other vital organs—HU may have to be started in very young children before there is irreversible renal medullary or organ damage. Such a study is now being planned. We do not yet know if HU will prevent organ damage in sickle cell disease or help restore function to already injured organs. Splenic regeneration has been reported in two adults with sickle cell anemia who had Hb F values of about 30% during HU treatment but the spleen may be a special case [32].

Baseline Hb F levels are lower in Hb SC disease than in sickle cell anemia. Increased cell volume and reduced cell density may occur independent of increases in Hb F in adults with Hb SC disease treated with HU [14]. Similar observations have been made in sickle cell anemia [11,13,33]. Two patients in the current study, both fe-

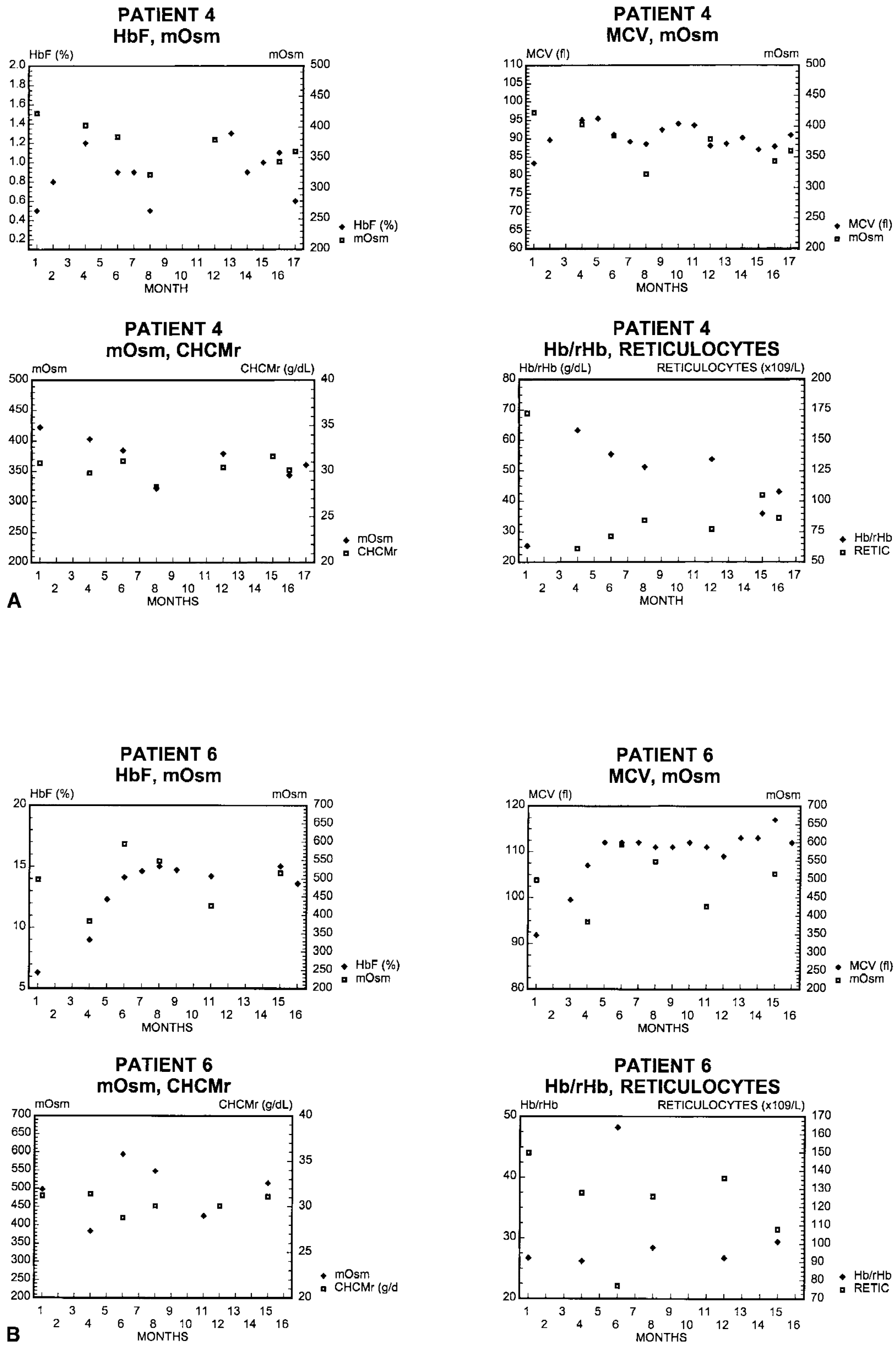

Fig. 1. Variations in maximal urine concentrating ability (mOsm), Hb F level, MCV, CHCMr, Hb/rHb, and reticulocyte count over time in two patients with Hb SC disease treated with HU. One patient (A) had little change in Hb F while the other had a rise in Hb F levels from 6.6% to 15% during treatment (B).

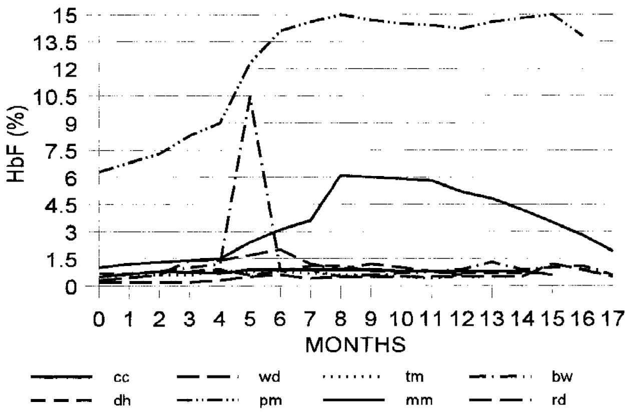

Fig. 2. Hb F levels during treatment with HU in eight children with Hb SC disease, mean age, 11 years.

male, increased their Hb F levels during treatment from 6% to 15% and from 1% to 6%, respectively. In our previous study, one man increased Hb F from 1.7% to 6.7% [14]. While the benefit of HU in sickle cell anemia may be due to factors beyond increased Hb F [13], perhaps higher doses of HU may be needed before a consistent rise in Hb F is observed in Hb SC disease.

Our data suggest that at the time we started our patients on HU, their ability to secrete concentrated urine was already compromised—perhaps irreversibly—due to structural damage to the countercurrent concentrating mechanism. Alternatively, we may have treated our patients for too short a time or the effects of this drug on the sickle cell may have been too small to allow restitution of the renal medullary circulation. Perhaps higher doses of drug given for a longer time would have proven effective.

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